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(54) **Rapid method for detection of methicillin-resistant staphylococci.**

(57) The present invention provides a method for the detection of methicillin-resistant staphylococci using the polymerase chain reaction. The reaction detects the presence of the mecA gene, which encodes penicillin binding protein 2A. Also provided is a method for the rapid release of DNA from staphylococci. The two methods can be used in combination for a rapid and sensitive route to the detection of these dangerous pathogens.

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Methicillin resistance in staphylococci is an important unversal problem in hospitals and geriatric care centers. Both methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant Staphylococcus epidermis (MRSE) are common nosocomial pathogens. The infections they cause are serious and difficult to treat. Horan et al., Morbid Mortal. Weekly Rep. 35:1755 (1984); Maple et al., Lancet i:537 (1989). Only a few antibiotics are available for treatment and they all have undesirable side effects. Hackbarth and Chambers, Antimicrob. Agents Chemother. 33:995 (1989). Currently, time-consuming, labor intensive, and somewhat unreliable methods are employed for the detection of MRSA/MRSE. Id These methods include disk diffusion, broth dilution and agar screening. Id Such methods do not reliably detect heterogeneously resistant staphylococci. Thus, it is imperative to develop a rapid, standardized, accurate and sensitive method for the detection of methicillin resistance in staphylococci.

MRSA/MRSE carry the mecA gene which encodes penicillin binding protein 2a (PBP2a). This proteins responsible for the phenotypic expression of methicillin resistance in staphylococci. Chambers, Antimicrob. Agents Chemother. 33:424 (1989); Hackbarth and Chambers, Antimicrob. Agents Chemother. 33:991 (1989); Tonin and Tomasz, Antimicrob. Agents. Chemother. 30:577 (1986). Staphylococcal strains susceptible to methicillin do not harbor a mecA gene. Therefore, the mecA, gene is a useful molecular handle for rapid identification of MRSA/MRSE. Detection of mecA by DNA hybridization has provided a relatively sensitive method for identifying MRSA/MRSE strains. Archer and Penell, Antimicrob. Agents Chemother. 34:1720 (1990); Lencastre et al., Antimicrob. Agents Chemother. 35:575 (1991). However, DNA hybridization suffers from several disadvantages. A large number of cells are required, DNA extraction and immobilization on a membrane is a time-consuming process, and frequently radioactive isotopes are employed, although recently non-radioactive probes have become available. Ligozzi et al., Antimicrob. Agents Chemother. 35:575 (1991).

The present invention provides a method for detecting methicillin-resistant staphylococcal infections, said method comprising:

a) performing the polymerase chain reaction on clinical samples suspected of staphylococcal infection, said polymerase chain reaction being primed by DNA primers composed of two oligonucleotides of high G+C content, wherein one oligonucleotide has a DNA sequence comprised by the coding strand of a Staphylococcus mecA gene and the second DNA primer has a DNA sequence comprised by the non-coding strand of a Staphylococcus mecA gene; and

b) analyzing the reaction product of step a.

The present invention also provides a method for the rapid release of DNA from staphylococci, said method comprising:

a) treating a sample containing staphylococci with lysostaphin;

b) treating the resultant sample of step a with proteinase K; and

c) incubating the resultant sample of step b in a boiling water bath.

Additionally, the present invention provides a method for detecting methicillin-resistant staphylococcal infections in a sample of interest, said method comprising:

a) treating a sample of interest with lysostaphin;

b) treating the resultant sample of step a with proteinase K;

c) incubating the resultant sample of step b in a boiling water bath;

d) performing the polymerase chain reaction on the resultant sample of step c, said polymerase chain reaction being primed by DNA primers, said DNA primers being composed of two oligonucleotides of high G+C content, wherein one oligonucleotide has a DNA sequence comprised by the coding strand of a Staphylococcus mecA gene and the second DNA primer has a DNA sequence comprised by the non-coding strand of a Staphylococcus mecA gene; and

e) analyzing the reaction product of step d.

For purposes of the present invention the following terms are defined below:

High G+C content - G+C content significantly higher than the average 30.5% G+C content of staphylococcal mecA genes.

mecA gene - a gene encoding penicillin binding protein 2A, which is responsible for methicillin resistance.

Methicillin-resistant staphylococci - staphylococci which are resistant to all beta-lactams, including cephalosporins and penicillin derivatives such as methicillin and oxacillin.

PBP2A - penicillin binding protein 2A.

The present invention provides a method for detecting methicillin-resistant staphylococcal infections, said method comprising:

a) performing the polymerase chain reaction on clinical samples suspected of staphylococcal infection, said polymerase chain reaction being primed by DNA primers, said DNA primers being composed of two oligonucleotides of high G+C content, wherein one oligonucleotide has a DNA sequence comprised by the coding strand of a Staphylococcus mecA gene and the second DNA primer has a DNA sequence comprised

by the non-coding strand of a Staphylococcus mecA gene; and

b) analyzing the reaction product of step a.

The polymerase chain reaction (PCR) is now well-known in the art as a route to the amplification of minute quantities of DNA. See, U.S. Patents 4,683,195, 4,800,159 and 4, 683,202. The technique has been applied in clinical settings to the detection of a variety of different pathogens. See, e.g., Cassol et al., J. Clin. Microbiol. 29:667-671 (1991); Lopez et al., J. Clin. Microbiol. 29:578-582 (1991); Brisson-Noel et al., Lancet ii:1069-1071 (1989); Valentine et al., J. Clin. Microbiol. 29:689-695 (1991); and Gouvea et al., J. Clin. Microbiol. 29:529-523 (1991). The technique has been used to detect toxins of Staphylococcus aureus but until the present invention the technique was not applied to the detection methicillin-resistant staphylococci.

Staphylococci resistant to beta-lactams, particularly Staphylococcus aureus and Staphylococcus epidermidis, represent a significant and steadily increasing problem for physicians. Due to the speed with which these bacteria can cause death, it is advantageous to quickly ascertain whether the organisms present in a patient are non-responsive to betalactams. The present invention provides a method which can be performed significantly faster than prior art methods (Hackbarth and Chambers, Antimicrob. Agents and Chemother. 33:995-999 (1989)), including methods based on DNA hybridization (Ligozzi et al., Antimicrob. Agents and Chemother. 35:575-578 (1991) and Archer and Pennel, Antimicrob. Agents and Chemother. 34:1720-1724 (1990)). The extreme sensitivity afforded by the PCR is another vital aspect of the present invention. The methicillin-resistant staphylococci are open heterogeneously resistant. Thus, non-DNA based resistance determinations can give inconsistent results.

Penicillin binding protein 2A confers methicillin resistance by having a low affinity for the beta-lactams. The proteins encoded by the mecA gene. The present invention will enable the detection of any staphylococci bearing the mecA gene. The clinically relevant staphylococcal strains are primarily S. epidermidis and S. aureus and, occasionally, S. haemolyticus. Rarely problematic strains are S. simulans, S. carnosus and S. saprophyticus. All the mecA genes which have been isolated and sequenced have a very high similarity (>99%), thus allowing the use of one or two sets of primers to detect all mecA-containing strains. If primers are made from regions of the gene which are known to contain variance among mecA genes isolated from different sources, the length of the primers used should be at least thirty nucleotides to ensure specific priming. These regions correlate to nucleotides 605-607, 615-617, 697-699, 746-747, 841, 1010-1011, 1819, and 1933 of SEQ ID NO:1.

The average G+C content of the known mecA genes is about 30.5%. To ensure specific priming, the G+C content of the DNA primers used for the PCR should be significantly higher than 30.5%, preferably about 50% or higher. The sequences of the DNA primers may be derived from any mecA gene. The sequences of some of these genes can be found in Song et al., FEBS Letters 221:167-171 (1987) (S. aureus TK784) and Ryffel et al., Gene 94:137-38 (1990) (S. aureus BB270 and S. epidermidis WT55). Additionally, the DNA primers may be derived from the S. aureus 27R mecA gene, set out as SEQ ID NO:1.

Skilled artisans will recognize that, within a particular set of primers, one of the oligonucleotides should be derived from the coding strand of the gene and the other oligonucleotide should be derived from the non-coding strand. Preferred DNA primers have sequences corresponding to nucleotides 141-160 of SEQ ID NO:1 and the inverse complement of nucleotides 1929-1952 of SEQ ID NO:1. The primers can be synthesized by the modified phosphotriester method using fully protected deoxyribonucleotide building blocks. Such synthetic methods are well known in the art and can be carried out in substantial accordance with Itakura et al., Science 198:1056 (1977), Crea et al., Proc. Nat'l Acad Sci. USA 75:5765 (1978), Hsiung et al., Nuc. Acids Res. 11:3227 (1983) or Narang et al., Methods in Enzymology 68:90. (1980). An especially preferred method employs automated DNA synthesizers such as the Applied Biosystems 380B DNA synthesizer (850 Lincoln Centre Drive, Foster City, CA 94404).

Protocols for performing the PCR reaction are set out in PCR Protocols: A Guide to Methods and Applications, ed. Michael A. Innis et al., Academic Press, Inc., 1990. An especially preferred protocol is described in the Examples herein. The results of the reaction may be analyzed in any way desired; a preferred method is by agarose gel electrophoresis. It may be desirable to further confirm the presence of staphylococci comprising the mecA gene by performing "nested PCR." In this technique, two consecutive PCRs are performed. The oligonucleotides used to prime the second PCR should be derived from DNA sequences of the mecA gene interior to the DNA sequences on which the first set of primers are based. Preferred DNA primers for the second PCR have sequences corresponding to nucleotides 568-593 of SEQ ID NO:1 and the inverse complement nucleotides 1647-1670 of SEQ ID NO:1.

The present invention also provides a method for the rapid release of DNA from staphylococci, said method comprising:

a) treating a sample containing staphylococci with lysostaphin;

b) treating the resultant sample of step a with proteinase K; and

c) incubating the resultant sample of step b in a boiling water bath. The method of the present invention is much faster than prior art staphylococcal DNA extraction methods. A preferred embodiment of the rapid release method is outlined in the Examples. The method is particularly useful in combination with the methicillin-resistant staphylococci detection technique of the present invention. Thus, the present invention also provides a method for detecting methicillin-resistant staphylococcal infections in a sample of interest, said method comprising:

a) treating a sample interest with lysostaphin;

b) treating the resultant sample of step a with proteinase K;

c) incubating the resultant sample of step b in a boiling water bath;

d) performing the polymerase chain reaction on the resultant sample of step c) said polymerase chain reaction being primed by DNA primers, said DNA primers being composed of two oligonucleotides of high G+C content, wherein one oligonucleotide has a DNA sequence comprised by the coding strand of a Staphylococcus mecA gene and the second DNA primer has a DNA sequence comprised by the non-coding strand of a Staphylococcus mecA gene; and

e) analyzing the reaction product of step d.

This method represents a combination of the rapid release DNA extraction method and the method for detecting methicillin-resistant staphylococci outlined above. The various considerations discussed regarding those two techniques are equally applicable when the methods are used in combination. The detection of methicillin-resistant staphylococci via the present invention requires only three hours. In contrast, when the prior art DNA extraction method is used in lieu of the rapid release DNA extraction method, six hours are required. Any additional time is significant in potential life or death situations such as methicillin-resistant staphylococcal infections. The speed and sensitivity of the present invention allows one to avoid empirical antibiotic therapy, which is fraught with liabilities such as high cost, toxicity factors and resistance development.

The present invention may be used to detect methicillin-resistant staphylococci in any internal body fluid, including blood, urine, spinal fluid and fluid drained from an abscess. If desired, the samples may be cultured and the PCR performed on a sample of the bacteria from the growth medium. The use of the PCR on body fluids is known in the prior art. See, e.g., Cassol et al., J. Clin. Microbiol. 29:667-671 (1991); Lopez et al., J.Clin. Microbiol. 29:578-582 (1991); and Brisson-Noel et al., Lancet ii:1069-1071 (1989).

The following Examples are intended to further illustrate and exemplify, but not limit the scope of, the present invention.

Example 1

A total of 70 staphylococcal strains (33 S. aureus and 37 S. epidermidis) isolated from various clinical settings were examined. Species identification was done with a Staph-Ident™ Diagnosic Kit (Analytab, Sherwood Medical, Plainview, NY). To verify the results obtained with the PCR, all were screened for methicillin resistance by growth on Mueller-Hinton agar (Difco, Detroit, MI) supplemented with 4% sodium chloride and 6 µg oxacillin/ml. Inoculated plates were incubated for 24 hours at 35 °C and examined for the presence of growth. Sixteen of 33 S. aureus and 27 of 37 S. epidermidis were categorized as methicillin-resistant using this method.

Example 2

Each strain examined for methicillin resistance in Example 1, above, was treated as follows. Bacteria were harvested from either TY agar plates (one loopful) or TY broth cultures (one ml of overnight culture diluted to 10^8 bacteria/ml). Cells from broth medium were harvested by centrifugation for 30 seconds in a microcentrifuge. Cells from either source were resuspended in 50 µl of lysostaphin solution (100 µg/ml in water, Sigma Chemical Co., St- Louis, MO). Cell suspensions were incubated at 37 °C. After 10 minutes, 50 µl proteinase K solution (100 µg/ml in water, Sigma), and 150 µl of 0.1 M Tris, pH 7.5, were added. The cell suspensions were incubated at 37 °C for an additional 20 minutes and then placed in a boiling water bath for 10 minutes. This treatment effectively lysed S. aureus or S. epidermidis cells and prevented DNase activity. Ten µl from these cell lysates were used directly in PCRs.

Example 3

Two primers were chosen for the PCR that were separated by 1.8 kb in the mecA open reading frame. The sequences of the two primers were 5'-GTTGTAGTTGTCGGGTTTGG-3'(nucleotides 141-160 of SEQ ID NO:1) and 5'-CCACCCAATTTGTCTGCCAGTTTCTCC-3'(inverse complement of nucleotides 1929-1952 of SEQ ID NO:1). The PCR was performed in a DNA Thermal Cycler using a Gene Amp Kit according to the manufacturer's

instructions (Perkin Elmer Cetus, Norwalk, CT). A thermal step program that included the following parameters was used for DNA amplification: denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, primer extension at 72 °C for two minutes, for a total of 30 cycles. Ten µl of the PCR solution were analyzed by electrophoresis on a 0.8% agarose gel. A positive result was indicated by the presence of a 1.8 kb amplified DNA fragment. In all, 69 of the 70 staphylococcal strains produced exactly the same result with PCR and the oxacillin susceptibility test. Examination of the single exception revealed that it was a mixed culture containng a rapidly growing methicillin-sensitive strain (mecA$^-$) and a slower growing methicillin-resistant (mecA$^+$) strain. When the strains were separated the susceptibility test and the PCR were in agreement. Subsequent nested PCR analysis of the intial DNA sample with the internal DNA primers (corresponding to nucleotides 568-593 of SEQ ID NO:1 and the inverse complement of nucleotides 1647-1670 of SEQ ID NO:1) produced a positive PCR result. No isolates were found to be mecA sensitive but methicillin resistant. These results emphasize the fact that a positive result in a DNA-based test correlates very well with the methicillin-resistant phenotype.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Eli Lilly and Company

(ii) TITLE OF INVENTION: Rapid Method for Detection of Methicillin Resistant Staphylococci

(iii) NUMBER OF SEQUENCES: 2

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(C) REFERENCE/DOCKET NUMBER: X-8573

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2111 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 105..2111

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ACACCTTCTA CACCTCCATA TCACAAAAAA TTATAACATT ATTTTGACAT AAATACTACA      60

TTTGTAATAT ACTACAAATG TAGTCTTATA TAAGGAGGAT ATTG ATG AAA AAG ATA      116
                                                Met Lys Lys Ile
                                                  1

AAA ATT GTT CCA CTT ATT TTA ATA GTT GTA GTT GTC GGG TTT GGT ATA      164
Lys Ile Val Pro Leu Ile Leu Ile Val Val Val Val Gly Phe Gly Ile
  5                  10                  15                  20

TAT TTT TAT GCT TCA AAA GAT AAA GAA ATT AAT AAT ACT ATT GAT GCA      212
Tyr Phe Tyr Ala Ser Lys Asp Lys Glu Ile Asn Asn Thr Ile Asp Ala
                 25                  30                  35

ATT GAA GAT AAA AAT TTC AAA CAA GTT TAT AAA GAT AGC AGT TAT ATT      260
Ile Glu Asp Lys Asn Phe Lys Gln Val Tyr Lys Asp Ser Ser Tyr Ile
             40                  45                  50

TCT AAA AGC GAT AAT GGT GAA GTA GAA ATG ACT GAA CGT CCG ATA AAA      308
Ser Lys Ser Asp Asn Gly Glu Val Glu Met Thr Glu Arg Pro Ile Lys
         55                  60                  65

ATA TAT AAT AGT TTA GGC GTT AAA GAT ATA AAC ATT CAG GAT CGT AAA      356
Ile Tyr Asn Ser Leu Gly Val Lys Asp Ile Asn Ile Gln Asp Arg Lys
     70                  75                  80

ATA AAA AAA GTA TCT AAA AAT AAA AAA CGA GTA GAT GCT CAA TAT AAA      404
Ile Lys Lys Val Ser Lys Asn Lys Lys Arg Val Asp Ala Gln Tyr Lys
 85                  90                  95                 100

ATT AAA ACA AAC TAC GGT AAC ATT GAT CGC AAC GTT CAA TTT AAT TTT      452
Ile Lys Thr Asn Tyr Gly Asn Ile Asp Arg Asn Val Gln Phe Asn Phe
                105                 110                 115

GTT AAA GAA GAT GGT ATG TGG AAG TTA GAT TGG GAT CAT AGC GTC ATT      500
Val Lys Glu Asp Gly Met Trp Lys Leu Asp Trp Asp His Ser Val Ile
            120                 125                 130

ATT CCA GGA ATG CAG AAA GAC CAA AGC ATA CAT ATT GAA AAT TTA AAA      548
Ile Pro Gly Met Gln Lys Asp Gln Ser Ile His Ile Glu Asn Leu Lys
        135                 140                 145

TCA GAA CGT GGT AAA ATT TTA GAC CGA AAC AAT GTG GAA TTG GCC AAT      596
Ser Glu Arg Gly Lys Ile Leu Asp Arg Asn Asn Val Glu Leu Ala Asn
    150                 155                 160

ACA GGA ACA GCA TAT GAG ATA GGC ATC GTT CCA AAG AAT GTA TCT AAA      644
Thr Gly Thr Ala Tyr Glu Ile Gly Ile Val Pro Lys Asn Val Ser Lys
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165                 170                 175                 180

AAA GAT TAT AAA GCA ATC GCT AAA GAA CTA AGT ATT TCT GAA GAC TAT      692
Lys Asp Tyr Lys Ala Ile Ala Lys Glu Leu Ser Ile Ser Glu Asp Tyr
                185                 190                 195

ATC AAA CAA CAA ATG GAT CAA AAT TGG GTA CAA GAT GAT ACC TTC GTT      740
Ile Lys Gln Gln Met Asp Gln Asn Trp Val Gln Asp Asp Thr Phe Val
            200                 205                 210

CCA CTT AAA ACC GTT AAA AAA ATG GAT GAA TAT TTA AGT GAT TTC GCA      788
Pro Leu Lys Thr Val Lys Lys Met Asp Glu Tyr Leu Ser Asp Phe Ala
        215                 220                 225

AAA AAA TTT CAT CTT ACA ACT AAT GAA ACA GAA AGT CGT AAC TAT CCT      836
Lys Lys Phe His Leu Thr Thr Asn Glu Thr Glu Ser Arg Asn Tyr Pro
    230                 235                 240

CTA GAA AAA GCG ACT TCA CAT CTA TTA GGT TAT GTT GGT CCC ATT AAC      884
Leu Glu Lys Ala Thr Ser His Leu Leu Gly Tyr Val Gly Pro Ile Asn
245                 250                 255                 260

TCT GAA GAA TTA AAA CAA AAA GAA TAT AAA GGC TAT AAA GAT GAT GCA      932
Ser Glu Glu Leu Lys Gln Lys Glu Tyr Lys Gly Tyr Lys Asp Asp Ala
                265                 270                 275

GTT ATT GGT AAA AAG GGA CTC GAA AAA CTT TAC GAT AAA AAG CTC CAA      980
Val Ile Gly Lys Lys Gly Leu Glu Lys Leu Tyr Asp Lys Lys Leu Gln
            280                 285                 290

CAT GAA GAT GGC TAT CGT GTC ACA ATC GTT GAC GAT AAT AGC AAT ACA     1028
His Glu Asp Gly Tyr Arg Val Thr Ile Val Asp Asp Asn Ser Asn Thr
        295                 300                 305

ATC GCA CAT ACA TTA ATA GAG AAA AAG AAA AAA GAT GGC AAA GAT ATT     1076
Ile Ala His Thr Leu Ile Glu Lys Lys Lys Lys Asp Gly Lys Asp Ile
    310                 315                 320

CAA CTA ACT ATT GAT GCT AAA GTT CAA AAG AGT ATT TAT AAC AAC ATG     1124
Gln Leu Thr Ile Asp Ala Lys Val Gln Lys Ser Ile Tyr Asn Asn Met
325                 330                 335                 340

AAA AAT GAT TAT GGC TCA GGT ACT GCT ATC CAC CCT CAA ACA GGT GAA     1172
Lys Asn Asp Tyr Gly Ser Gly Thr Ala Ile His Pro Gln Thr Gly Glu
                345                 350                 355

TTA TTA GCA CTT GTA AGC ACA CCT TCA TAT GAC GTC TAT CCA TTT ATG     1220
Leu Leu Ala Leu Val Ser Thr Pro Ser Tyr Asp Val Tyr Pro Phe Met
            360                 365                 370

TAT GGC ATG AGT AAC GAA GAA TAT AAT AAA TTA ACC GAA GAT AAA AAA     1268
Tyr Gly Met Ser Asn Glu Glu Tyr Asn Lys Leu Thr Glu Asp Lys Lys
        375                 380                 385

GAA CCT CTG CTC AAC AAG TTC CAG ATT ACA ACT TCA CCA GGT TCA ACT     1316
Glu Pro Leu Leu Asn Lys Phe Gln Ile Thr Thr Ser Pro Gly Ser Thr
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     390                 395                 400

CAA AAA ATA TTA ACA GCA ATG ATT GGG TTA AAT AAC AAA ACA TTA GAC      1364
Gln Lys Ile Leu Thr Ala Met Ile Gly Leu Asn Asn Lys Thr Leu Asp
405                 410                 415                 420

GAT AAA ACA AGT TAT AAA ATC GAT GGT AAA GGT TGG CAA AAA GAT AAA      1412
Asp Lys Thr Ser Tyr Lys Ile Asp Gly Lys Gly Trp Gln Lys Asp Lys
                425                 430                 435

TCT TGG GGT GGT TAC AAC GTT ACA AGA TAT GAA GTG GTA AAT GGT AAT      1460
Ser Trp Gly Gly Tyr Asn Val Thr Arg Tyr Glu Val Val Asn Gly Asn
            440                 445                 450

ATC GAC TTA AAA CAA GCA ATA GAA TCA TCA GAT AAC ATT TTC TTT GCT      1508
Ile Asp Leu Lys Gln Ala Ile Glu Ser Ser Asp Asn Ile Phe Phe Ala
        455                 460                 465

AGA GTA GCA CTC GAA TTA GGC AGT AAG AAA TTT GAA AAA GGC ATG AAA      1556
Arg Val Ala Leu Glu Leu Gly Ser Lys Lys Phe Glu Lys Gly Met Lys
    470                 475                 480

AAA CTA GGT GTT GGT GAA GAT ATA CCA AGT GAT TAT CCA TTT TAT AAT      1604
Lys Leu Gly Val Gly Glu Asp Ile Pro Ser Asp Tyr Pro Phe Tyr Asn
485                 490                 495                 500

GCT CAA ATT TCA AAC AAA AAT TTA GAT AAT GAA ATA TTA TTA GCT GAT      1652
Ala Gln Ile Ser Asn Lys Asn Leu Asp Asn Glu Ile Leu Leu Ala Asp
                505                 510                 515

TCA GGT TAC GGA CAA GGT GAA ATA CTG ATT AAC CCA GTA CAG ATC CTT      1700
Ser Gly Tyr Gly Gln Gly Glu Ile Leu Ile Asn Pro Val Gln Ile Leu
            520                 525                 530

TCA ATC TAT AGC GCA TTA GAA AAT AAT GGC AAT ATT AAC GCA CCT CAC      1748
Ser Ile Tyr Ser Ala Leu Glu Asn Asn Gly Asn Ile Asn Ala Pro His
        535                 540                 545

TTA TTA AAA GAC ACG AAA AAC AAA GTT TGG AAG AAA AAT ATT ATT TCC      1796
Leu Leu Lys Asp Thr Lys Asn Lys Val Trp Lys Lys Asn Ile Ile Ser
    550                 555                 560

AAA GAA AAT ATC AAT CTA TTA ACT GAT GGT ATG CAA CAA GTC GTA AAT      1844
Lys Glu Asn Ile Asn Leu Leu Thr Asp Gly Met Gln Gln Val Val Asn
565                 570                 575                 580

AAA ACA CAT AAA GAA GAT ATT TAT AGA TCT TAT GCA AAC TTA ATT GGC      1892
Lys Thr His Lys Glu Asp Ile Tyr Arg Ser Tyr Ala Asn Leu Ile Gly
                585                 590                 595

AAA TCC GGT ACT GCA GAA CTC AAA ATG AAA CAA GGA GAA ACT GGC AGA      1940
Lys Ser Gly Thr Ala Glu Leu Lys Met Lys Gln Gly Glu Thr Gly Arg
            600                 605                 610

CAA ATT GGG TGG TTT ATA TCA TAT GAT AAA GAT AAT CCA AAC ATG ATG      1988
Gln Ile Gly Trp Phe Ile Ser Tyr Asp Lys Asp Asn Pro Asn Met Met
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          615                 620                 625

ATG GCT ATT AAT GTT AAA GAT GTA CAA GAT AAA GGA ATG GCT AGC TAC      2036
Met Ala Ile Asn Val Lys Asp Val Gln Asp Lys Gly Met Ala Ser Tyr
    630                 635                 640

AAT GCC AAA ATC TCA GGT AAA GTG TAT GAT GAG CTA TAT GAG AAC GGT      2084
Asn Ala Lys Ile Ser Gly Lys Val Tyr Asp Glu Leu Tyr Glu Asn Gly
645                 650                 655                 660

AAT AAA AAA TAC GAT ATA GAT GAA TA                                   2111
Asn Lys Lys Tyr Asp Ile Asp Glu
                665
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 668 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Lys Lys Ile Lys Ile Val Pro Leu Ile Leu Ile Val Val Val Val
 1               5                  10                  15

Gly Phe Gly Ile Tyr Phe Tyr Ala Ser Lys Asp Lys Glu Ile Asn Asn
            20                  25                  30

Thr Ile Asp Ala Ile Glu Asp Lys Asn Phe Lys Gln Val Tyr Lys Asp
        35                  40                  45

Ser Ser Tyr Ile Ser Lys Ser Asp Asn Gly Glu Val Glu Met Thr Glu
    50                  55                  60

Arg Pro Ile Lys Ile Tyr Asn Ser Leu Gly Val Lys Asp Ile Asn Ile
65                  70                  75                  80

Gln Asp Arg Lys Ile Lys Lys Val Ser Lys Asn Lys Lys Arg Val Asp
                85                  90                  95

Ala Gln Tyr Lys Ile Lys Thr Asn Tyr Gly Asn Ile Asp Arg Asn Val
            100                 105                 110

Gln Phe Asn Phe Val Lys Glu Asp Gly Met Trp Lys Leu Asp Trp Asp
        115                 120                 125

His Ser Val Ile Ile Pro Gly Met Gln Lys Asp Gln Ser Ile His Ile
    130                 135                 140

Glu Asn Leu Lys Ser Glu Arg Gly Lys Ile Leu Asp Arg Asn Asn Val
145                 150                 155                 160
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Glu Leu Ala Asn Thr Gly Thr Ala Tyr Glu Ile Gly Ile Val Pro Lys
165 170 175

Asn Val Ser Lys Lys Asp Tyr Lys Ala Ile Ala Lys Glu Leu Ser Ile
180 185 190

Ser Glu Asp Tyr Ile Lys Gln Gln Met Asp Gln Asn Trp Val Gln Asp
195 200 205

Asp Thr Phe Val Pro Leu Lys Thr Val Lys Lys Met Asp Glu Tyr Leu
210 215 220

Ser Asp Phe Ala Lys Lys Phe His Leu Thr Thr Asn Glu Thr Glu Ser
225 230 235 240

Arg Asn Tyr Pro Leu Glu Lys Ala Thr Ser His Leu Leu Gly Tyr Val
245 250 255

Gly Pro Ile Asn Ser Glu Glu Leu Lys Gln Lys Glu Tyr Lys Gly Tyr
260 265 270

Lys Asp Asp Ala Val Ile Gly Lys Lys Gly Leu Glu Lys Leu Tyr Asp
275 280 285

Lys Lys Leu Gln His Glu Asp Gly Tyr Arg Val Thr Ile Val Asp Asp
290 295 300

Asn Ser Asn Thr Ile Ala His Thr Leu Ile Glu Lys Lys Lys Lys Asp
305 310 315 320

Gly Lys Asp Ile Gln Leu Thr Ile Asp Ala Lys Val Gln Lys Ser Ile
325 330 335

Tyr Asn Asn Met Lys Asn Asp Tyr Gly Ser Gly Thr Ala Ile His Pro
340 345 350

Gln Thr Gly Glu Leu Leu Ala Leu Val Ser Thr Pro Ser Tyr Asp Val
355 360 365

Tyr Pro Phe Met Tyr Gly Met Ser Asn Glu Glu Tyr Asn Lys Leu Thr
370 375 380

Glu Asp Lys Lys Glu Pro Leu Leu Asn Lys Phe Gln Ile Thr Thr Ser
385 390 395 400

Pro Gly Ser Thr Gln Lys Ile Leu Thr Ala Met Ile Gly Leu Asn Asn
405 410 415

Lys Thr Leu Asp Asp Lys Thr Ser Tyr Lys Ile Asp Gly Lys Gly Trp
420 425 430

Gln Lys Asp Lys Ser Trp Gly Gly Tyr Asn Val Thr Arg Tyr Glu Val
435 440 445

Val Asn Gly Asn Ile Asp Leu Lys Gln Ala Ile Glu Ser Ser Asp Asn
450 455 460

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Ile Phe Phe Ala Arg Val Ala Leu Glu Leu Gly Ser Lys Lys Phe Glu
465                 470                 475                 480

Lys Gly Met Lys Lys Leu Gly Val Gly Glu Asp Ile Pro Ser Asp Tyr
                485                 490                 495

Pro Phe Tyr Asn Ala Gln Ile Ser Asn Lys Asn Leu Asp Asn Glu Ile
            500                 505                 510

Leu Leu Ala Asp Ser Gly Tyr Gly Gln Gly Glu Ile Leu Ile Asn Pro
        515                 520                 525

Val Gln Ile Leu Ser Ile Tyr Ser Ala Leu Glu Asn Asn Gly Asn Ile
    530                 535                 540

Asn Ala Pro His Leu Leu Lys Asp Thr Lys Asn Lys Val Trp Lys Lys
545                 550                 555                 560

Asn Ile Ile Ser Lys Glu Asn Ile Asn Leu Leu Thr Asp Gly Met Gln
                565                 570                 575

Gln Val Val Asn Lys Thr His Lys Glu Asp Ile Tyr Arg Ser Tyr Ala
            580                 585                 590

Asn Leu Ile Gly Lys Ser Gly Thr Ala Glu Leu Lys Met Lys Gln Gly
        595                 600                 605

Glu Thr Gly Arg Gln Ile Gly Trp Phe Ile Ser Tyr Asp Lys Asp Asn
    610                 615                 620

Pro Asn Met Met Met Ala Ile Asn Val Lys Asp Val Gln Asp Lys Gly
625                 630                 635                 640

Met Ala Ser Tyr Asn Ala Lys Ile Ser Gly Lys Val Tyr Asp Glu Leu
                645                 650                 655

Tyr Glu Asn Gly Asn Lys Lys Tyr Asp Ile Asp Glu
            660                 665
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Claims

1. A method for detecting methicillin resistant staphylococcal infections, said method comprising:
   a) performing the polymerase chain reaction on clinical samples suspected of staphylococcal infection, said polymerase chain reaction being primed by DNA primers, said DNA primers being composed of two oligonucleotides of high GC content, wherein one oligonucleotide has a DNA sequence comprised by the coding strand of a Staphylococcus mecA gene and the second DNA primer has a DNA sequence comprised by the non-coding strand of a Staphylococcus mecA gene; and
   b) analyzing the reaction product of step a.

2. The method of Claim 1 wherein the GC content of the DNA primers is approximately 50% or greater.

3. The method of Claim 2 wherein the sequences of the DNA primers are nucleotides 141-160 of SEQ ID NO:1 and the inverse complement of nucleotidas 1929-1952 of SEQ ID NO:1.

4. The method of Claim 1 wherein an additional subsequent polymerase chain reaction is performed using DNA primers interior to the DNA primers used in the initial polymerase chain reaction.

5. The method of Claim 4 wherein the sequences of the interior DNA primers are nucleotides 568-593 of SEQ ID NO:1 and the inverse complement of nucleotides 1647-1670 of SEQ ID NO:1.

6. The method of Claim 1 wherein the sequence of the DNA primers is comprised by the coding and non-coding strands of a gene selected from a group consisting of Staphylococcus aureus and Staphylococcus epidermidis mecA genes.

7. The method of Claim 6 wherein the gene is selected from a group consisting of the S. aureus 27R, S. aureus BB270, S. aureus TK784, and the S. epidermidis WT55 mecA genes.

8. The method of Claim 1 wherein the method is used to detect methicillin resistant staphylococci selected from the group consisting of S. aureus, S. epidermidis, S. haemolyticus, S. simulans, S. carnosus, and S. saprophyticus.

9. The method of Claim 1 wherein the reaction product of step a) is analyzed by gel electrophoresis.

10. A method for the rapid release of DNA from staphylococci, said method comprising:
    a) treating a sample containing staphylococci with lysostaphin;
    b) treating the resultant sample of step a with proteinase K; and
    c) incubating the resultant sample of the step in a boiling water bath.

11. The method of Claim 10 wherein the sample containing staphylococci is derived from blood, urine, spinal fluid, an abscess or bacteriological growth medium.

12. A method for detecting methicillin resistant staphylococcal infections in a sample of interest, said method comprising:
    a) treating a sample of interest with lysostaphin;
    b) treating the resultant sample of step a with proteinase K;
    c) incubating the resultant sample of step b in a boiling water bath;
    d) performing the polymerase chain reaction on the resultant sample of step c) said polymerase chain reaction being primed by DNA primers, said DNA primers being composed of two oligonucleotides of high GC content, wherein one oligonucleotide has a DNA sequence comprised by the coding strand of a Staphylococcus mecA gene and the second DNA primer has a DNA sequence comprised by the non-coding strand of a Staphylococcus mecA gene; and
    e) analyzing the reaction product of step d.

13. The method of Claim 12 wherein the GC content of the DNA primers is approximately 50% or greater.

14. The method of Claim 13 wherein the sequences of the DNA primers are nucleotides 141-160 of SEQ ID NO:1 and the inverse complement of nucleotides 1929-1952 of SEQ ID NO:1.

15. The method of Claim 14 wherein an additional subsequent polymerase chain reaction is performed using DNA primers interior to the DNA primers used in the initial polymerase chain reaction.

16. The method of Claim 15 wherein the sequences of the interior DNA primers are nucleotides 568-593 of SEQ ID NO:1 and the inverse complement of nucleotides 1647-1670 of SEQ ID NO:1

17. The method of Claim 12 wherein the sequence of the DNA primers is comprised by the coding and non-coding strands of a gene selected from a group consisting of Staphylococcus aureus and Staphylococcus epidermidis mecA genes.

18. The method of Claim 17 wherein the sequence of the DNA primers is comprised by the coding and non-coding stands of a gene selected from a group consisting of the S. aureus 27R, S. aureus BB270, S. aureus TK784, and the S. epidermis WT55 mecA genes.

19. The method of Claim 12 wherein the method is used to detect methicilin-resistant staphylococci selected from the group consisting of S. aureus, S. epidermidis, S. haemolyticus, S. simulans, S. carnosus, and S. saprophyticus.

20. The method of Claim 12 wherein the reaction product of step d) is analyzed by gel electrophoresis.

21. The method of Claim 12 wherein the sample of interest is derived from blood, urine, spinal fluid, an abscess or bacteriological growth medium.

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EUROPEAN SEARCH REPORT

Application Number

EP 92307307.6

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
P,A	WO - A - 92/05 281 (CHUGAI SEIYAKU KABUSHIKI KAISHA) * Abstract; claims 3-13,16 * --	1,3,5, 12,14, 16	C 12 Q 1/68 C 07 H 21/00 C 12 Q 1/14 //(C 12 Q 1/14 C 12 R 1:445
A	WO - A - 91/08 305 (U-GENE RESEARCH B.V.) * Abstract; claims * --	1,3,5, 12,14, 16	C 12 R 1:45)
A	CHEMICAL ABSTRACTS, vol. 102, no. 15, April 15, 1985 Columbus, Ohio, USA W.F. NAUSCHUETZ et al. "Rapid detection of oxacillin-resistant staphylococci using an automated system." page 335, abstract-no. 128 675r & J. Med. Technol 1985, 2(1), 60-2 --		
A	CHEMICAL ABSTRACTS, vol. 102, no. 15, April 15, 1985 Columbus, Ohio, USA A.P. BARTON "A rapid bioluminescent method for the detection of methicillin-resistant Staphylococcus aureus colonies." pages 335-6, abstract-no. 128 684t & J. Antimicrob. Chemother. 1985, 15(1), 61-7 --		
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TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C 12 Q
C 07 H

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	10-11-1992	SCHNASS

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
	-resistant staphylococci with the MS-2 system." page 329, abstract-no. 3 832n & Can. J. Microbiol. 1984, 30(4), 488-90 ----		

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

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